REMARKS

The Office Action

The Combined Declaration and Power of Attorney is objected to. The specification is objected to for containing browser-executable code.

Claims 1-18 are pending in this application. All pending claims stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. Claims 1-11, 17, and 18 stand further rejected under 35 U.S.C. § 112, second paragraph for indefiniteness. Claims 1-11 stand rejected under 35 U.S.C. § 102(e) in view of U.S. Patent 6,284,539.

Combined Declaration and Power of Attorney

A newly executed Combined Declaration and Power of Attorney form is enclosed herewith. This objection can now be withdrawn.

Objection to the Specification

All browser-executable code has been deleted from the specification by amendment. This objection can now be withdrawn.

Rejections Under 35 U.S.C. § 112, first paragraph

All pending claims stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. The Examiner applies a three-part rejection. First, the Examiner asserts that "[t]he specification fails to provide an enabling disclosure for the genetic modification of human ES cells" because success in mouse ES cells is not predictive of success in human ES cells. Second, the Examiner asserts that the specification does not teach how to produce a therapeutic effect using the claimed methods. Third, the Examiner asserts that the specification fails to enable a method for transplantation. Applicants respectfully disagree with these bases of rejection and address each separately below.

Genetic Modification of Human ES Cells

The Examiner asserts that the specification is not enabling because successful genetic modification of mouse ES cells is not predictive of success in human ES cells. To support this rejection, the Examiner points out that Zwaka *et al.* (*Nature Biotechnol.* Advance Online Publication, February 10, 2003; "Zwaka") recently demonstrated significant differences in transfection efficiencies between mouse and human ES cells. The Examiner also notes a recent study by Eiges *et al.* (*Curr. Biol.* 11: 514-518, 2001; "Eiges") which compares the efficiency of different transfection protocols for human ES cells. The Examiner argues that, together, these studies prove that methods for successfully transfecting human ES cells were not available at the time of application filing. Applicants respectfully disagree.

Applicants respectfully submit that the Examiner's reading of Zwaka is unduly narrow and does not demonstrate that the present specification is non-enabling. The Examiner characterizes Zwaka as teaching that "[h]igh, stable transfection efficiencies in human ES cells have been difficult to achieve, and, in particular, electroporation protocols established for mouse ES cells work poorly in human ES cells." *Office Action* mailed February 26, 2003, page 4, fifth paragraph; emphasis added.

Applicants first point out that mere inefficiency is not an appropriate basis for a lack of enablement rejection. In the statement relied upon by the Examiner, Zwaka refers to transfecting human ES cells using protocols that had been established and optimized for mouse ES cells. Zwaka discloses the mouse ES cell protocols yielded a stable transfection rate of about 10^{-7} in human ES cells. *Zwaka*, page 1, right column, first paragraph. Although Zwaka suggests that murine-optimized electroporation protocols work poorly in human ES cells and that higher transfection rates are desirable, nowhere does Zwaka suggest that electroporation of human ES cells is entirely unsuccessful. Thus, for this reason alone, Zwaka proves that the instant specification enables the genetic modification of human ES cells.

Zwaka also demonstrates that, by performing nothing more than routine experimentation, the murine electroporation protocols may be optimized for human ES cells and higher yields obtained. Specifically, Zwaka states that

> [a]s human ES cells are significantly larger than mouse ES cells (~14 μm versus ~8 μm), we tried electroporation parameters described for larger cells. Additionally, we electroporated the cells in an isotonic, protein-rich solution (standard cell culture medium), instead of PBS, at room temperature. Using this modified protocol, we were able to obtain stable ... transfection rates that were 100-fold (or more) higher than those attained with standard mouse ES cell electroporation procedures. Zwaka at page 1, right column, first paragraph.

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> [e]lectroporation of human ES cells with a DNA construct containing a *neo* cassette under the control of the *tk* promoter yielded a stable transfection rate of 5.6×10^{-5}, giving an estimated 26:1 ratio of stable transfected clones to homologous recombination events for the first POU5F1 construct. Similarly, for transfection of the HPRT1 vector, the ratio of G418-resistant clones to $HPRT1^-$ clones was 50:1. Zwaka at page 2, first paragraph (citation omitted) (emphasis added).

Zwaka, using standard techniques known in the art at the time of application filing, easily modified the murine-optimized electroporation protocols to increase transfection efficiency in human ES cells.

Zwaka further provides the artisan with an expectation of success by teaching that the recombination event is likely equivalent in mouse and human ES cells. Zwaka notes that "although successful transfection strategies differ between human and mouse ES cells, the frequency of homologous recombination itself may be similar." Zwaka at page 2, first paragraph; emphasis added.

In sum, Zwaka demonstrates that the results of murine-optimized electroporation protocols are successful, albeit inefficient, when applied to human ES cells. Zwaka further demonstrates that the protocols are easily improved by routine experimentation using techniques known in the art for transfection of larger ES cells (i.e., human). And

finally, Zwaka confirms that the process of homologous recombination is similarly efficient in human and murine ES cells (not that efficiency is relevant to enablement under § 112). Accordingly, when read in its entirety, Zwaka alone demonstrates the viability of creating genetically modified human ES cells using the electroporation technique and proves that Applicants' specification enabled genetic modification of human ES cells at the time of filing.

Even accepting, *arguendo*, the Examiner's position that Zwaka demonstrates the requirement for undue experimentation to successfully electroporate human ES cells at the time of application filing, Eiges proves that alternative techniques for genetic modification of human ES cells were available to the skilled artisan. Using human ES cells, Eiges compares the transfection efficiency of several common protocols and reagents. Specifically, Eiges compares three chemical transfection reagents (Lipofectamine, FuGENE, and Exgen 500 (polyethylenimine)) and electroporation. Eiges, like Zwaka, demonstrate that electroporation of human ES cells is successful (Eiges, Figure 1). Eiges also demonstrates that transfection of human ES cells using FuGENE (Boehringer Mannheim) and ExGen 500 (Fermentas, Inc.) is successful.

Applicants note that murine ES cells were successfully transfected with a vector encoding Nurr-1 using Lipofectamine according to the manufacturer's protocol (Example 2, 9, and 10). Thus, as evidenced by Eiges, chemical transfection methods similar to the one used by Applicants can be successfully applied to human ES cells. (See, for example, Remy *et al. Adv. Drug Deliv. Rev.* 30: 85-95, 1998; Uyttersprot *et al. Mol. Cell. Endocrinol.* 142: 35-39, 1998; Wiesenhofer *et al. J. Neurosci. Meth.* 92: 145-152, 1999; copies enclosed).

In sum, Zwaka and Eiges support, rather than refute, Applicants' assertion that a skilled artisan at the time of application filing, performing no more than routine experimentation, was able to genetically modify human ES cells. Furthermore, Applicants also point out that Eiges was published on April 3, 2001; a date prior to the filing of the instant application. Accordingly, the art cited by the Examiner proves that

the specification, combined with techniques and reagents available at the time of filing, enables the genetic modification of human ES cells, making this an improper basis to support a lack of enablement rejection.

Therapeutic Effects of Transplantation

The Examiner asserts that the specification does not enable the treatment of Parkinson's Disease (PD) and raises several technical issues including assertions that (i) gene therapy is unpredictable, (ii) the specification does not teach how to produce replacement neurons at the critical locations, and (iii) there are insufficient teachings as to the technical aspects of the method including "the level of gene expression required, the number of transduced cells needed, when or for how long the gene should be expressed, [and] the frequency of administration of the transfected neuronal precursor cells required."

Each of these bases of rejection are addressed individually below. Applicant's point out, however, that the claims, as presently amended, do not encompass transplantation of embryonic stem cells. Rather, the claimed method transplants primarily dopaminergic neurons that are derived from embryonic stem cells.

Applicants' Invention Is Not Gene Therapy

In rejecting all pending claims, the Examiner asserts that

> [t]he specification fails to teach an appropriate method for transferring a recombinant cell comprising a cell fate-inducing gene and expressing that gene at a level necessary to produce the desired therapeutic effect in a diseased animal, i.e. to produce replacement neurons at the critical locations. *Office Action*, page 4, lines 1-4.

In support of this basis of rejection, the Examiner also points out that

> [a]t the time the application was filed, the art of administering any type of genetic expression vector, including transfected cells, to an individual so as to

provide a tangible therapeutic benefit was poorly developed and unpredictable. *Office Action*, page 4, lines 11-13.

Applicants point out that the presently claimed method is a cell therapy, not a gene therapy, and it does not depend upon *in vivo* gene expression. The expression of a cell fate-inducing gene by the ES cell is merely to promote differentiation along a dopaminergic cell fate pathway and is performed primarily in culture prior to transplantation. As demonstrated in Figure 5B (also see Example 11, page 32), Nurr-1 expression *in vitro* urges a higher proportion of cultured ES cells to adopt a dopaminergic phenotype, compared to cultured naïve ES cells.

The dopaminergic-inducing effect of expressing a cell-fate inducing gene *in vitro* is complemented by transplantation of the ES cell into the brain. As demonstrated by Deacon *et al.* (Blastula-stage stem cells can differentiate into dopaminergic and serotonergic neurons after transplantation. *Exp. Neurol.* 149: 28-41, 1998; art of record) and in Example 13 (page 35), naïve ES cells differentiate along a dopaminergic cell fate pathway upon transplantation into the brain. Thus, even if not all transplanted cells are differentiated (i.e., some ES cells remain), the transplanted ES cells are highly likely to differentiate *in vivo* regardless of whether they express a cell fate-inducing gene. Contrary to the Examiner's assertion, it is the cell, not the gene, which confers the therapeutic effect and, because the phenotypic change in the ES cell can be monitored *in vitro* prior to transplantation, no guidance on *in vivo* gene expression is required.

Transplanted Cells Produce Replacement Neurons

The Examiner next argues that "the state of the art for *in vivo* differentiation of ES cells is undeveloped" and that "little is known about the behavior of these cells *in vivo* or how they will interact with the local environment when implanted into adult tissues." *Office Action*, page 5. Applicants respectfully disagree. However, as noted previously, the claims have been amended to primarily encompass transplantation of dopaminergic neurons derived from ES cells. Because the claimed method involves transplanting

neurons rather than ES cells, enabling guidance is found in the prior art. For example, fetal neurons isolated from the ventral mesencephalon (VM cells) contain, *inter alia*, dopaminergic neurons. Nonetheless, at the time of application filing, it was recognized that VM cell grafts implanted into the human putamen[1] alleviated the clinical symptoms caused by PD and innervate the surrounding brain tissue. Lindvall *et al. Ann. Neurol.* 31:155-165, 1992 (art of record); Linvall, *Mov. Disord.* 13(Suppl. 1): 83-87, 1998 (copy enclosed).

When read together, the instant specification, Lindvall *et al.*, and Lindvall demonstrate that the dopaminergic neurons derived from ES cells according to the present invention, and fetal VM cells (including fetal dopaminergic neurons), adopt neuronal phenotypes and innervate the surrounding nervous tissue when transplanted into the damaged striatum (caudate, in humans). Further and as noted below, the evidence from human PD patients and non-human animal PD models (including the 6-OH-DA model) prove the effectiveness of dopaminergic cell replacement therapies for the treatment of PD. Thus, for these reasons alone, the presently claimed method of treating PD using dopaminergic neurons derived from ES cells is fully enabled by the specification.

In addition to being enabling for the use of ES cell-derived dopaminergic neurons, the specification also demonstrates that naïve ES cells, should any be present, spontaneously adopt a neuronal phenotype when transplanted into the brain (see Examples 12-14). Dopaminergic differentiation of naïve ES cells was observed in two different rodent models of Parkinson's disease—the MPTP mouse model (Examples 12 and 13) and the 6-hydroxydopamine rat model (Examples 14 and 15) . When viewed together with the results of Example 3 and Figure 5, demonstrating *in vitro* dopaminergic differentiation of Nurr-1-expressing ES cells, the skilled artisan immediately recognizes that the transgenic ES cells are likely to differentiate into dopaminergic neurons in a higher proportion than naïve ES cells. Thus, the instant specification provides sufficient guidance for the skill artisan to centrally transplant Nurr-1-expressing ES cells in a

1 The caudate and putamen of primates (i.e., humans) is equivalent to the corpus striatum of rodents.

manner that would result in ES cell dopaminergic differentiation and innervate the surrounding nervous tissue.

Applicants' assertions of the therapeutic effectiveness of Nurr-1-expressing ES cells for treating dopaminergic deficits has been independently verified by Kim *et al.* (*Nature* advance online publication, 20 June 2002; copy enclosed). Kim *et al.* transplanted Nurr-1-expressing ES cells into the striatum of rats unilaterally lesioned using 6-hydroxydopamine (6-OH-DA). The transplanted ES cells adopted a dopaminergic phenotype as measured by electrophysiology and immunohistochemistry. Additionally, the behavioral symptoms of the 6-OH-DA lesion, a surrogate for the symptoms of PD in humans, were reversed. Thus, the study of Kim *et al.*, using lineage restricted, Nurr-1-expressing ES cells equivalent to the genetically modified ES cells described in the present specification, provides an expectation that the claimed method will be successful and demonstrates that the specification fully enables the claimed invention.

The specification, along with pre- and post-filing evidence, fully enables the claimed method. In sum, Applicants demonstrate that dopaminergic neurons may be derived *in vitro* from genetically modified ES cells which, using standard techniques, may be transplanted into the PD brain. Even if the transplanted cell population is not entirely differentiated (i.e., some naïve or recombinant ES cells are present), the ES cell precursors are likely to contribute to the therapeutic efficacy of the composition. Thus, replacement neurons are produced at critical locations by (i) direct transplantation of *in vitro* ES cell-derived dopaminergic neurons, and (ii) *in vivo* differentiation of naïve and modified ES cells.

Technical Aspects of the Gene Expression and Cell Transplantation

The Examiner further asserts that there are insufficient teachings on the technical aspects of the invention including

> the level of gene expression required, the number of transduced cells needed, when or for how long the gene should be expressed, or the frequency of administration of the transfected neuronal precursor cells required, for the treatment of any pathological condition. *Office Action*, page 4, lines 8-11.

Applicants respectfully disagree. In Examples 2, 3, and 10, Applicants clearly describe experimental conditions for the genetic modification of murine ES cells using a Nurr-1-containing plasmid, and *in vitro* culture conditions that promote dopaminergic differentiation. Figure 5B is a photomicrograph of Nurr-1-expressing ES cells that exhibit molecular markers of dopaminergic neurons. As discussed above, Applicants submit that it requires no more than routine experimentation to adapt the transfection and culture conditions for murine ES cells for use with human ES cells.

The specification provides significant teachings on the number of ES cells that should be used for transplantation. Applicants respectfully direct the Examiner's attention to Example 13; an experiment characterizing the effect of low density and high density ES cell grafts on dopaminergic differentiation in the MPTP mouse model of Parkinson's disease. In this experiment, Applicants engrafted 50,000, 2,000, or 200 naïve ES cells into the lesioned striatum and discovered that the best result was achieved with the lowest density graft. Specifically, grafts containing 200 ES cells resulted in an average of 1250 dopaminergic neurons and an absence of tumor-like formations. The 2,000 ES cells grafts produced fewer dopaminergic neurons while the 50,000 ES cell grafts produced tumor-like structures—an adverse effect. Thus, Applicants have demonstrated that low density ES cell grafts are superior to high density ones. Here again, only routine experimentation is required to optimize the number of ES cells or dopaminergic neurons that, when engrafted into to a human brain, will give the most beneficial result.

Applicants also demonstrate the therapeutic efficacy of ES cell transplants. In Examples 14 and 15, and Figure 7, Applicants demonstrate that naïve ES cell grafts containing 1000-2000 cells are sufficient to reduce the functional deficits caused by dopaminergic denervation in the 6-hydroxydopamine model of Parkinson's disease.

Specifically, amphetamine-induced turning (a functional indicator of a unilateral dopaminergic deficit) is reduced in rats 7 and 9 weeks after transplantation of the ES cells into the lesioned striatum.

Taken together, the data presented in the specification provides the skilled artisan with ample guidance on methods for making the genetically modified ES cells, culture conditions, and transplantation parameters including the number of cells to be engrafted and an indication of the expected onset of therapeutic benefit. The rodent models used in these experiments are well validated in the prior art as surrogates for Parkinson's disease in humans.

Methods for Transplantation of Embryonic Stem Cells

Finally, the Examiner asserts that

> [t]he specification fails to provide an enabling disclosure for the method of transplantation because methods of transplantation of neural tissue are not routinely successful and the specification does not offer adequate guidance to enable one skilled in the art to practice the claimed invention to derive a therapeutic benefit in a diseased animal. *Office Action*, page 5, lines 3-6.

Applicants respectfully disagree. Insofar as this basis of rejection applies to the expectation that the transplantation of ES cells provides effective therapy for Parkinson's disease, the Examiner is directed to Examples 14 and 15, and Figure 7 (discussed above), which demonstrate that transplants containing naïve ES cells attenuate functional deficits caused by dopaminergic denervation, similar to that observed in the pathological Parkinson's brain. Furthermore, Kim *et al.* (*supra*) independently verify that ES cells expressing Nurr-1, when transplanted into the lesioned nervous system, are also capable improving electrophysiological, biochemical, and functional indices of dopaminergic damage.

Methods for cell graft transplantation into the human brain are well documented in the prior art. The Examiner is directed to the teachings of Olle Lindvall, for example

(Update on Fetal Transplantation: The Swedish Experience, *Movement Disorders*, 13(Suppl. 1): 83-87, 1998). Dr. Lindvall characterizes the state of the art of human neural grafting. He notes that "[s]ince the first clinical trials in 1987, a total of more than 200 patients with PD worldwide have received implants of human fetal mesencephalic tissue into the striatum" (page 83; emphasis added). Thus, Dr. Lindvall makes clear that transplantation techniques for humans are widely available and long term graft viability is routine. The Examiner has provided no evidence to suggest that techniques for transplanting fetal mesencephalic tissue cannot be used for transplanting ES cells into the human brain.

In sum, Applicants respectfully submit that, at the time of application filing, a skilled artisan, practicing no more than routine experimentation, could have created the genetically-modified ES cells used in the present invention. The genetically-modified ES cells, when transplanted into the brain of a PD patient, form replacement neurons in critical locations and adopt the appropriate phenotype necessary to correct the deficiency, as disclosed in the specification. For the foregoing reasons, Applicants submit that the specification fully enables the practice of the claimed invention and this rejection should be withdrawn.

Rejections Under 35 U.S.C. § 112, second paragraph

Claims 1-11, 17, and 18 stand further rejected under 35 U.S.C. § 112, second paragraph for indefiniteness. The Examiner asserts that the term "cell fate-inducing genes" is indefinite. This rejection has been overcome by the present claims amendments and may be withdrawn.

Rejections Under 35 U.S.C. § 102

Claims 1-11 stands rejected under 35 U.S.C. § 102(e) as anticipated by U.S. Patent 6,284,539 ("the '539 patent). Specifically, the Examiner points out that the '539 patent discloses introducing a Nurr-1 gene into CNS (neural) stem cells, causing them to adopt a

dopaminergic fate, and then using those cells for transplantation to treat Parkinson's disease. Applicants note that the instant claims have been amended to encompass only the use of embryonic stem cells which are different from the neural stem cells of the '539 patent. Accordingly, this rejection is overcome by the present amendments and should be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is requested. Enclosed is a petition to extend the period for replying for three months, to and including April 6, 2004. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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